

Karyotyping and single-gene detection using fluorescence in situ hybridization on chromosomes of *Hydra magnipapillata* (Cnidaria: Hydrozoa)

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Abstract. The fresh water polyp *Hydra* L., 1758 (Cnidaria, Hydrozoa) plays a key role as a model organism in modern evolutionary and developmental biology. A complete genome sequence has been published recently for *Hydra magnipapillata* Ito, 1947 and molecular data are rapidly accumulating in the literature, but little information is available on its chromosomes. In this study, an efficient fluorescence *in situ* hybridization (FISH) method is described for *H. magnipapillata* which not only allows identification of the chromosomes but also visualization of the location of individual genetic loci. Together with cDNA and genomic sequencing this may provide the foundation for increasingly precise genetic and physical mapping in this basal metazoan model organism.

Key words: *Hydra magnipapillata*, karyotype, chromosomes, fluorescence *in situ* hybridization (FISH), single-gene detection, Qdots.

INTRODUCTION

Freshwater hydras (Cnidaria, Hydrozoa, *Hydra* L., 1758) have long been of general interest since they display fundamental principles that underlie development, differentiation, regeneration and symbiosis (e.g. Bosch et al., 2010; Augustin et al., 2010; Bosch 2007, 2008; Khalturin et al., 2009). The sequencing of the *Hydra magnipapillata* Ito, 1947 genome recently has shed light on the evolution and development of complexity of multicellular animals (Chapman et al., 2010) and revealed that these simple multicellular organisms have developed many of the molecular switches that are required for the differentiation of higher organisms. Despite its alleged simplicity, *H. magnipapillata* has a large, complex

genome of 1.0 to 1.5 billion base pairs (Zacharias et al., 2004; Chapman et al., 2010) and around 20,000 protein-encoding genes. The number of genes in *H. magnipapillata* is considerably higher than that in *Drosophila* and slightly lower than the number of genes in highly developed organisms such as human, mouse, and pufferfish (*Tetraodon nigroviridis* Marion de Proce, 1822). Similarities between *H. magnipapillata* and other metazoan species extend beyond gene number and gene product sequence, to include intron-exon structure, higher order chromatin arrangements typical of mammalian cell nuclei (Alexandrova et al., 2003), and conserved genome structure (synteny / gene linkage) (Chapman et al., 2010). However, while molecular data on

Hydra species are rapidly accumulating in the literature (e.g. Bosch et al., 2009; Khalturin et al., 2009; Chandramore et al., 2010; Gee et al., 2010; Hartl et al., 2010; Munder et al., 2010), little information is available on their chromosomes.

This is to be regretted because considerable information can be obtained by examining karyotypes: for example, sex determination depends in many cases on the presence or absence of sex chromosomes that may be morphologically differentiated. Comparing chromosomes in different species may also shed light on the systematics and evolution of these organisms and provide an even deeper understanding not only of *Hydra*'s evolutionary history, but also of the structural changes that have shaped genome evolution in this group of basal metazoans. Previous studies have shown that the number of chromosomes is identical in all *Hydra* species examined ($2n = 30$). The size of the chromosomes is strictly correlated with the size of the genome, with *Hydra viridissima* Pallas, 1766 having conspicuously small chromosomes (Zacharias et al., 2004). One of the conserved chromosome features is telomere molecular organization: in basal metazoans including *Hydra vulgaris* as well as in vertebrates the telomeres consist of a highly conserved telomeric repeat motif (TTAGGG) $_n$ (Traut et al., 2007). Whether maintenance of *Hydra*'s telomeres by telomerase activity is responsible for *Hydra*'s remarkable immortality (Martinez, 2002) and stem cells which continuously proliferate and thereby generate eternal lineages (Wittlieb et al., 2006; Bosch, 2009) is not known. Studies on longevity and senescence in the jellyfish *Cassiopea spp.* have uncovered telomerase activity in somatic tissues of both the polyp and medusa stages (Ojimi et al., 2009). In several species of coral telomere lengths were greater than 19 kb (Zielke, Bodnar, 2010).

Karyotypes in the genus *Hydra* are poorly understood. Only nine of about 30 *Hydra* species have been karyologically studied so far. In the majority of cases, karyological data are restricted to chromosome numbers. Previously, methods of classical cytogenetics and differential staining techniques did not reveal clear differences in banding patterns of *Hydra* chromosomes (Xinbai et al., 1987; Ovanesyan, Kuznetsova, 1995; Anokhin, Kuznetsova, 1999; Anokhin, 2002, 2004; Anokhin, Nokkala, 2004; Zacharias et al., 2004). The aim of this study was, therefore, to develop fluorescence *in situ* hybridization (FISH) to characterize chromosomes in *Hydra magnipapillata*. In this paper we demonstrate that the "vertebrate" telomere motif (TTAGGG) $_n$ was conserved at the end of each *H. magnipapillata* chromosome. Localization of a number of candidate genes including *18S rDNA*, *28S rDNA*, *Tol2*, *DMRT* and *ks1* on distinct chromosome pairs was studied and not only demonstrated the utility of FISH for identifying chromosomes in this species but also provided first evidence that individual genetic loci can be visualized in *Hydra magnipapillata*. In future this may aid in assembling physical and genetic maps in this widely used model organism.

MATERIAL AND METHODS

Specimens

Chromosome and FISH analysis were carried out with *Hydra magnipapillata* (strain 105). The animals were cultured according to standard conditions at $18 \pm 0.5^\circ\text{C}$.

Chromosome preparations

FISH was performed on mitotic plates from cells of asexual *H. magnipapillata* polyps. Chromosome preparations were obtained using the air-drying method: polyps were treated in a hypotonic 0.4 % sodium citrate solution

for 25 min and then fixed in 3:1 (v/v) ethanol-glacial acetic acid for 15–30 min. Fixed polyps were homogenized in 0.1–0.3 ml of 70 % acetic acid. The cell suspension was dropped on prewarmed (37–40°C) cleaned slides and dried at 37–40°C.

Chromosome staining

Chromosomes were stained as described by Anokhin, Nokkala (2004).

DNA isolation, PCR amplification, cloning and sequencing, probe generation

Genomic DNA from 100 specimens was isolated using a standard Phenol/Chloroform nucleic acid extraction protocol. FISH was carried out on hydra chromosomes using different probes. The target probes were PCR amplified using heterologous primers designed for *Hydra magnipapillata*. *28SrDNA* primers: 28SrRNA_F 5'-GCTAAGCTTTGACGAGTAGG-3', 28SrRNA_R 5'-CTGCCACAAGCCAGTTATC-3' (1520 bp fragment); *18SrDNA* primers: 18SrRNA_F 5'-GATCCTGCCAGTAGTCATATG-3', 18SrRNA_R 5'-GAGTCAAATTAAGCCG-CAGG-3' (1186 bp fragment). *ks1* primers: Ks1-1_F 5'-AAGCTAATAATTGTGCTAG-TAATGAT-3'; Ks1-1_R 5'-TGCTTCTTTTATCTTTGAGGTTTTTT-3' (1500 bp fragment). *TOL2* Transposable elements probe was amplified using M13_F/R primers from Kiel-1 BAC-library (clone tad60e07.y²) (1500 bp fragment). *DMRT* primers: HyDMRT_F 5'-GAAACTTGTGATGAACAGGCATC-3', DMRT_R 5'-GGAGAGATTAGAACA-CAAACCCAG-3' (1621 bp fragment). TTAGGG telomere motif was PCR amplified using primers: TTAGGG_F 5'-CCCTAAC-CCTAACCTAACCTAACCTAA-3' and TTAGGG_R 5'-TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG-3'.

Resulting PCR fragments (with the exception of telomeric probe) were cloned into pGEM-T vector (Promega,

Madison, Wisconsin) and transformed into electrocompetent DH10B *E. coli* cells (Invitrogen, Karlsruhe, Germany). Plasmids were sequenced using a LI-COR 4300 DNA Analyzer plate sequencer (LI-COR Biosciences, Lincoln, Nebraska).

DMRT sequence has been submitted to GenBank (accession No. HQ687211).

BAC clones containing the *18S rRNA* gene (BAC 16C18) and BAC clones without ribosomal genes (BAC 1K4 PPOD, BAC 42P3 PPOD, BAC 16E21, BAC 18A7 ks-1) were selected and picked out from Kiel BAC-libraries. BAC DNA was isolated according to the Qiagen Plasmid Midi Kit Protocol.

The *18S rDNA*, *28S rDNA*, *ks1*, *TOL2* transposable elements genes and telomeric probes were labeled with biotin or digoxigenin by PCR. BAC probes were labeled by random primer labeling with biotin or digoxigenin according to the manufacturer's (Roche) instructions.

Fluorescence in situ hybridization (FISH) analysis of Hydra magnipapillata chromosomes

In situ hybridization was performed as described by Schwarzscher and Heslop-Harrison (2000) with modifications. Chromosome preparations were treated with 100 µg/ml RNaseA (Sigma-Aldrich, Moscow, Russia) for 50 min at 37°C in a humid chamber, washed twice in 2x SSC (5 min each) at 37°C, incubated in 0.01% pepsin in 0.01 N HCl for 10 min at 37°C, washed in 1x PBS for 1 min at RT and in 2x SSC for 5 min at 37°C, dehydrated through an ice cold ethanol series (70%, 90% and 99%, 2 min each) and finally, dried. After pretreatment, preparations were mounted using frames for *in situ* hybridization (Peqlab, Erlangen, Germany) at 40°C with 90 µl predenatured (5 min at 96°C) hybridization solution containing 150–200 ng of labeled genomic DNA, 25% formamide, 4x SSC, 10%

(w/v) dextran sulfate, 0.15% (w/v) sodium dodecyl sulfate (SDS) and 2 µg salmon-sperm DNA or/and 10 µg sonicated in 250-500 bp fragments *H. magnipapillata* DNA. The slides were placed in a prewarmed histological table and denatured for 5 min at 68–69°C. The temperature was gradually reduced to 37–40°C, and then the chromosome slides were incubated for 42–44 h at 37°C. Following hybridization, the slides were washed three times in 2x SSC (5 min each) at 37°C, three times in 0.5x SSC (3 min each) at 43°C, incubated in detection buffer (4x SSC/ 0.1–0.2% Tween) for 2 min at 37°C and blocked in 2% (w/v) BSA/ 4x SSC/0.2% Tween for 25 min at 37°. Probes were detected with 10.0–13.0 µg/ml avidin or streptavidin conjugated to FITC or rhodamine (Sigma-Aldrich, Moscow, Russia). The detection reaction was performed in 2 % BSA/ 4x SSC/ 0.1% Tween for 1 h at 37°C. Slides were washed three times in 4x SSC/ 0.1% Tween (5 min each) at 37°C and rinsed in 1x PBS at 37°C. All washes were static. Chromosomes were contrasted with 1 µg/ml DAPI and mounted in an antifade solution containing 60% Glycerol in 1x PBS and 5% DABCO (1,4-diazabicyclo[2.2.2]octane; Sigma-Aldrich, Moscow, Russia).

An alternative protocol for *in situ* hybridization with *DMRT* gene and telomeric repeats was used also: chromosome preparations were dehydrated through 70/80/96% ethanol at RT and treated with 100 µg/ml RNaseA (Sigma-Aldrich, Moscow, Russia) for 60 min at 37°C in humid chamber; washed three times in 2x SSC (5 min each) at RT; dehydrated through 70/80/96% ethanol at RT; incubated in 5 mg/ml Pepsin in 0.01 N HCl for 15 min at 37°C; washed sequentially in 1x PBS, in PBSx1/0.05M MgCl₂ for 5 min each, in 1% PFA in PBSx1/0.05M MgCl₂ for 10 min, in 1x PBS for 5 min, in PBSx1/0.05M MgCl₂ for 5 min at RT each; dehydrated

through 70/80/96% ethanol at RT or at ice cold and finally, dried. After pretreatment hybridization mixture containing about 100 ng of labeled probe, 50% formamide, 2xSSC, 10% (w/v) dextran sulfate, 1% (w/v) Tween-20 and 10 µg salmon-sperm DNA was added on preparations. Slides were mounted using glass coverslips and rubber cement. The slides were denatured for 5 min at 75°C. Then the chromosome slides were incubated for 42–44 h at 37°C. Following hybridization, the slides were washed in 2x SSC for 3 min at 45°C, then in 50% formamide in 2x SSC for 10 min at 45°C, two times in 2x SSC (10 min each) two times in 0.2x SSC (10 min each) at 45°C, blocked in 1.5% (w/v) BSA/4x SSC/0.1% Tween-20 (5% (w/v) BSA/1x PBS/0.1% Tween-20 before Qdots detection) for 30 min at 37° in humid chamber. Probes were detected with 5 µg/ml Avidin-Alexa Fluor 488 (Invitrogen, Moscow, Russia) or 10 nM Qdots Streptavidin Conjugate 655 (Invitrogen, Moscow, Russia). The detection reaction was performed in 1.5 % BSA/ 4x SSC/ 0.1% Tween-20 (1.5 % BSA/ 1xPBS/ 0.1% Tween-20 for Qdots detection) for 1 h at 37°C. Slides were washed three times in 4x SSC/ 0.02% Tween-20 (or in 1xPBS after Qdots detection) for 10 min each at 45° and dehydrated through 70/80/96% Ethanol at RT. Chromosomes were mounted in a mounting-antifade (ProLong Gold antifade reagent with DAPI, Invitrogen, Moscow, Russia) and cover with a glass coverslip.

Microscopy and imaging

Microscopic images were taken using a Zeiss Axioskop 2 with a 100x objective and a Zeiss AxioCam HR camera and merged with Adobe Photoshop or using Leica DM 4000B and a Leica DFC 350 FX camera using a Leica Application Suite 2.8.1 software with an Image Overlay module (Leica).

Southern blot analysis

About 20 µg of isolated DNA was digested

with 4 Units of restrictases HindIII and XbaI respectively. Nucleic acids were transferred to Hybond N+ nylon membranes (Amersham, Biosciences). Hybridizations were carried out over night in Church buffer at 55 °C followed by washes in 0.2x SSC/ 0.1 % SDS at room temperature, 42 °C and 60 °C for 2x 30 minutes, depending on the signal/ background ratio. Autoradiographies were performed using phosphoimaging plates and the phosphoimager FLA-5000 (FUJI). DNA-probes were radiolabeled with P-[32P]-dCTP using the Megaprime DNA labeling System (Amersham Biosciences).

RESULTS AND DISCUSSION

Basic chromosome features in Hydra magnipapillata

The diploid karyotype includes 30 chromosomes (Fig. 1). The karyotype is symmetric. The largest chromosome pair bears an achromatic gap in every homologue. The centromere positions are generally difficult to distinguish after conventional staining. After DAPI staining followed by C-banding procedure (Fig. 1, D), blocks of constitutive heterochromatin were found only in the centromere regions of the chromosomes. All chromosomes are two-armed, meta- and submetacentric (Fig. 1, C, D). No heteromorphic chromosome pair (sex chromosomes) could be identified (Fig. 1). Conventional staining techniques including HOECHST- and Quinacrine- staining did not provide markers for identification of individual chromosomes (Fig. 1, A, B). Since chromosomes in *H. magnipapillata* represent a regular gradation in size, a preliminary *H. magnipapillata* karyogram could be produced displayed in decreasing order of size (Fig. 1, E).

Fluorescence in situ hybridization (FISH)

and chromosomal mapping

Fluorescence *in situ* hybridization (FISH) allows identification of the location and abundance of a DNA sequence to be determined by hybridization of a labeled DNA probe to chromosomes and nuclei (Schwarzacher, Heslop-Harrison, 2000). Previously FISH studies in *Hydra vulgaris* revealed highly repetitive DNA in telomere positions (Traut et al., 2007). To establish FISH in *H. magnipapillata* and to ascertain the conserved nature of telomeres within the genus *Hydra*, we have carried out FISH using a (TTAGGG)_n probe to test the presence of TTAGGG telomere repeat sequences on *H. magnipapillata* chromosomes. As shown in Figure 2, signals are visible on all chromosomes ends. The results match those in *H. vulgaris* (Traut et al., 2007) and support the view that chromosomes are capped by highly conserved telomeres.

For chromosome mapping we used random BAC probes which do not include *rDNA* genes. Unexpectedly, numerous signals with variable patterns of localization in different mitotic plates were revealed with every BAC probe, whereas single spots were expected. One examples of such hybridization pattern is shown in Fig. 3, A. We speculate that this is most probably due to the presence of abundant repetitive elements in the BAC fragments that can hybridize with complimentary sequences on the chromosomes. Because the abundance of dispersed repetitive elements appears to prevent the direct use of the currently available *Hydra* BACs as FISH probes, we next tested Tol2-like transposable element probes as a chromosome marker in *Hydra magnipapillata*. Southern blot hybridization data (Fig. 3, B) showed the presence of multiple copies of a Tol2 transposable element gene in the *H. magnipapillata* genome. Consistent with this observation, FISH hybridization with

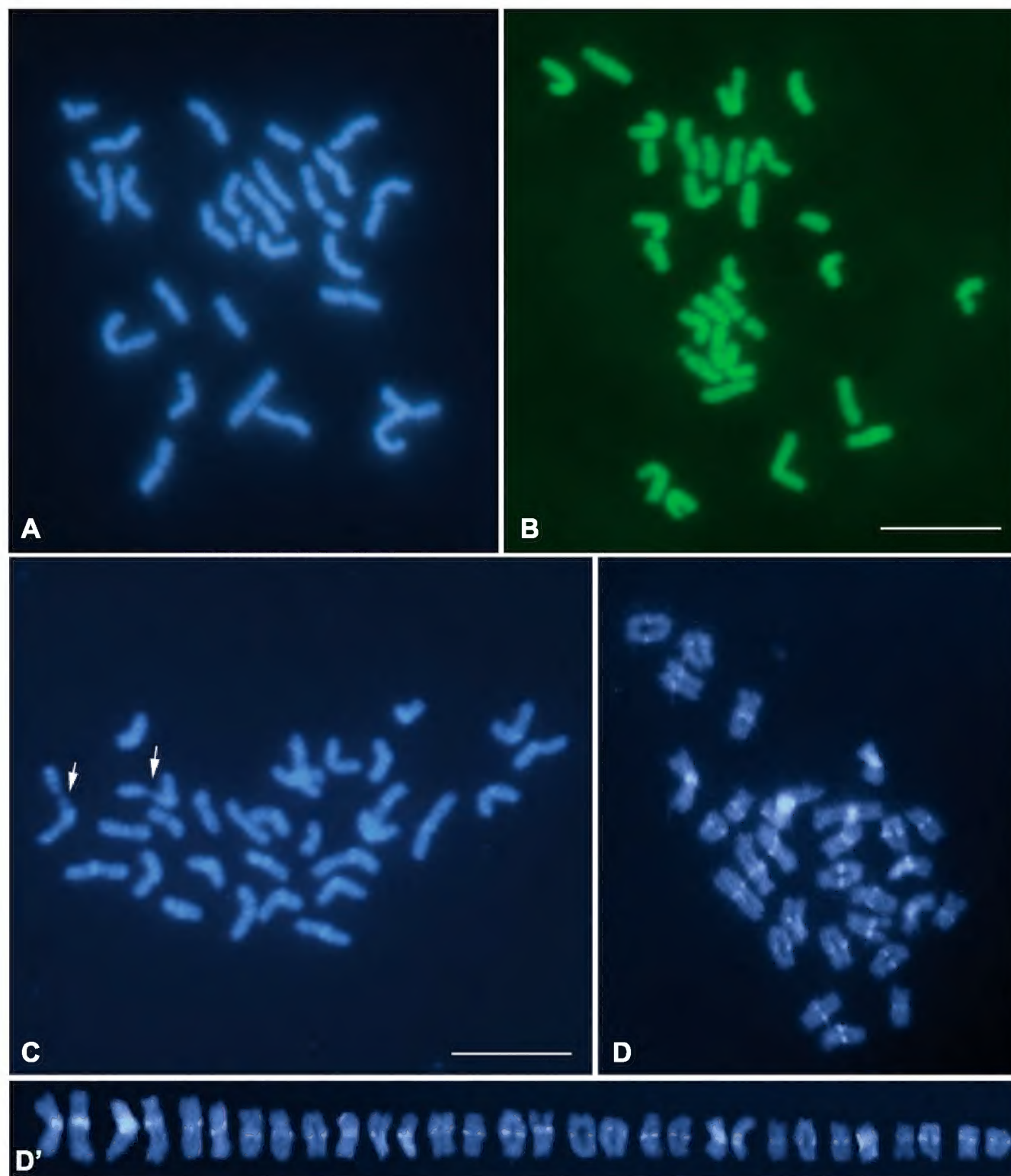


Fig. 1, A-D'. Mitotic plates of *Hydra magnipapillata* after HOECHST staining (A) and Quinacrine staining (B). No banding pattern is visible. Mitotic plates of *Hydra magnipapillata* after DAPI staining (C) and C-banding/DAPI staining (D, D'). DAPI-positive signals are sometimes visible in the centromeres; arrows indicate gaps in the 1st chromosome pair; (D') Karyogram. Bars = 10 μ m.

these probes revealed non-identical patterns of numerous signals in the chromosomes (Fig. 3 C, D).

Fluorescence in situ hybridization (FISH) using rDNA and single-gene probes

As alternative to universal markers we next examined the hybridization behavior of a number of selected candidate genes lacking highly repetitive elements. After performing

FISH with both ribosomal *18S rDNA* and *28S rDNA* probes, including BAC-probes (BAC 16C18) containing *18S rDNA* fragment, specific signals were localized on a single chromosome pair (Fig. 4) which consists of hydra's largest chromosomes and, therefore, is designated pair #1. The signals obtained correspond to achromatic gaps revealed by routine methods of chromosome staining.

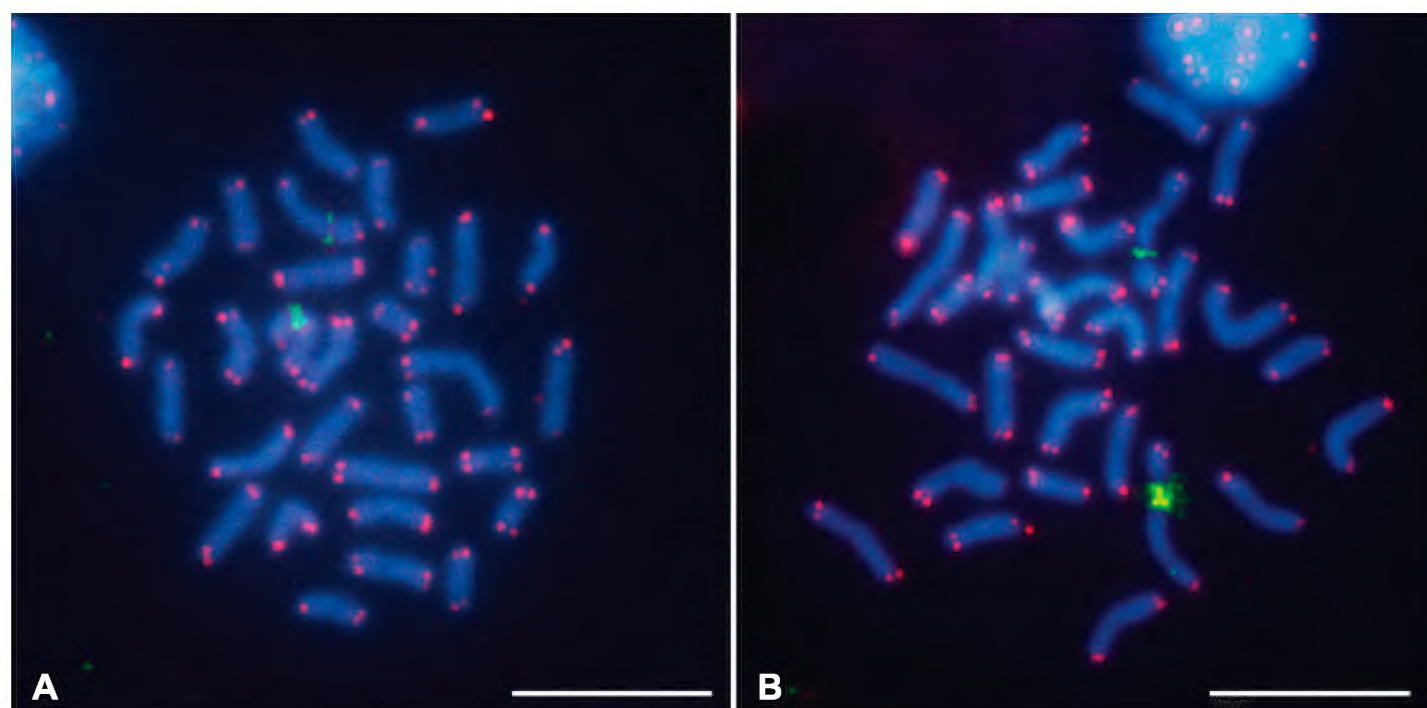


Fig. 2, A, B. FISH in *H. magnipapillata* mitotic chromosomes with telomere (red signals) and 18S rDNA (green signals) probes. The telomere signals are visible on all chromosomes ends. Two different mitotic plates are shown (A, B). Chromosomes are counterstained blue with DAPI. Bars = 10 μ m.

FISH location of ks1 genes on H. magnipapillata chromosomes

Head-specific gene *ks1* is sensitive to patterning signals along the apical–basal body axis of *Hydra* and regulated by a complex interaction of inhibitory factors (Weinziger et al., 1994; Endl et al., 1999). *ks1* loss-of-function polyps have defects specifically during head regeneration, but not foot regeneration, indicating that this gene is functionally involved in head development (Lohmann et al., 1999). To localize *ks1* on *H. magnipapillata* chromosomes, FISH using both the 1.5 kb genomic fragment of *H. magnipapillata ks1* gene (Weinziger et al., 1994) and the 10 kb BAC clone 18A7 containing the *ks1* gene (Hemmrich, Bosch, unpubl.) resulted in *ks1*-specific signals on three pairs of chromosomes (Fig. 5). In some experiments few additional signals were visible. Since *ks1* belongs to a rather complex gene family (Hemmrich, Bosch, unpubl.) this probably is the result of hybridization of the *ks1* probe with similar sequences of other *ks1* gene family sites. After hybridization with BAC clone 18A7

containing *ks1* gene sequence (Fig. 5 C, D) we detected additional weak hybridization signals on nearly all chromosomes. This is most probably due to hybridization of additional genomic flanking sequences of this BAC probe with corresponding sequences on *Hydra*'s chromosomes. Based on morphology and size of the chromosomes and the localization of *ks1* the *H. magnipapillata* chromosomes, we constructed the karyograms shown in Figure 5.

FISH Location of a DMRT1-related gene on a single chromosome pair

In the *Hydra magnipapillata* karyotype, no heteromorphic pair of chromosomes could be detected indicating the absence of sex chromosomes. Since in some cases (Marín et al., 2000; Vicoso, Bachtrog, 2009) morphologically distinct sex chromosomes can be traced back to an initially identical chromosome pair, it seems possible that *Hydra* have a pair of morphologically identical chromosomes bearing sex-linked genes (“sex chromosomes”) or a pair of autosomes with clusters of sex linked genes. To address this

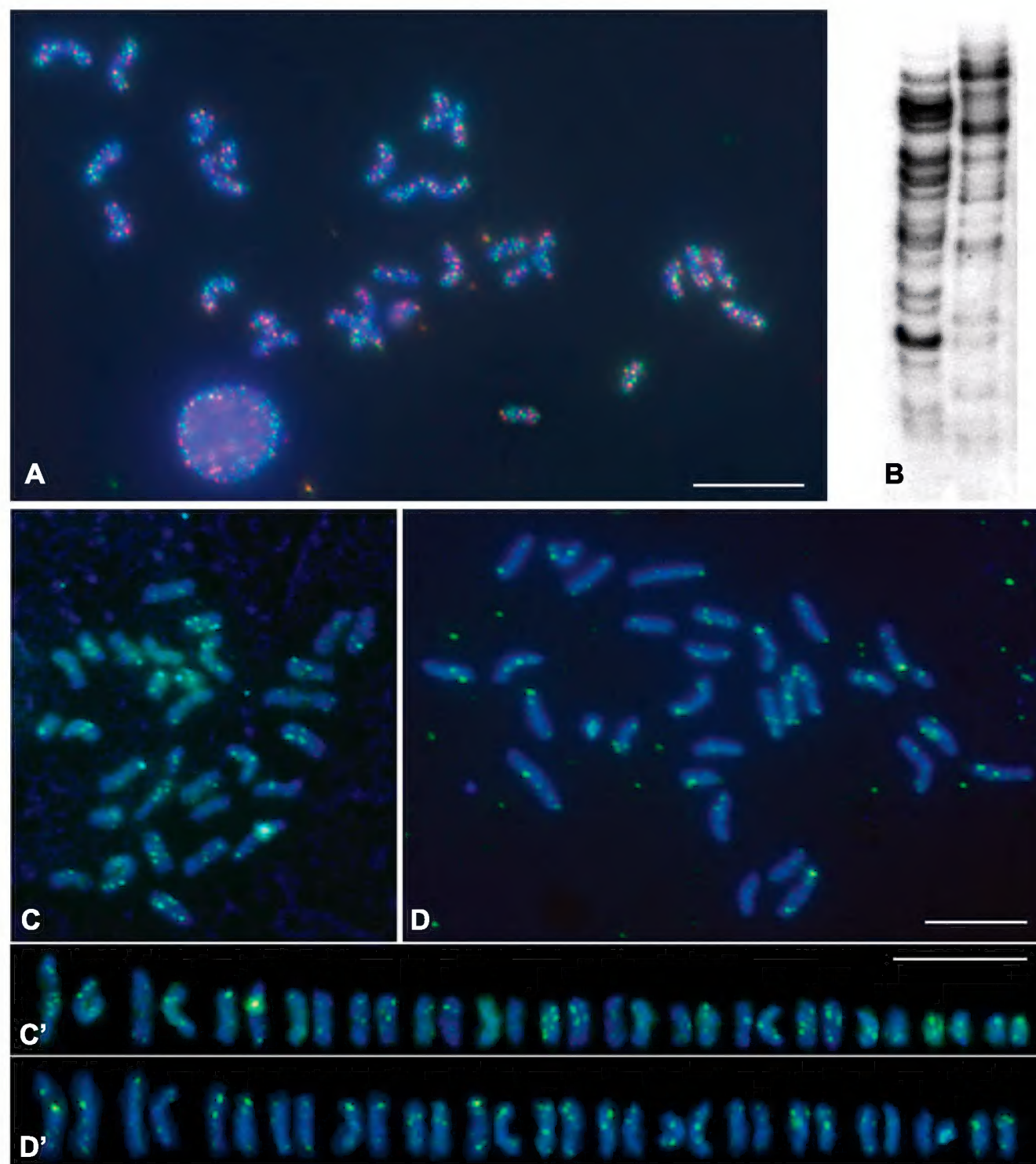


Fig. 3, A-D'. FISH in *H. magnipapillata* mitotic chromosomes with two BAC probes. Numerous signals are visible in every chromosome (A). Southern blot of DNA sample of *H. magnipapillata* digested with HindIII (left lane) and EcoRI (right lane) hybridized with *Tol2* transposable element probe (B). FISH in *H. magnipapillata* mitotic chromosomes with *Tol2* transposable element probe. Signals are visible in every chromosome (C-D). Mitotic plates (C, D) and karyograms (C', D') from different FISH experiments. Chromosomes are counterstained blue with DAPI. Bars = 10 μ m.

question, as a first step we decided to isolate a *H. magnipapillata* member of the *DMRT* gene family of transcription factors and to determine its chromosomal localization. *DMRT* genes appear to represent the only factors involved in sexual development that are conserved across the phylum Chordata (Winkler et al., 2004). The first known and prototype member,

DMRT1, is implicated in vertebrate male development, although with some species-specific differences. Invertebrate counterparts implicated in sex determination and differentiation include *Drosophila* doublesex (*dsx*) and the *Caenorhabditis elegans* *Mab3* gene (Winkler et al., 2004).

To isolate a *DMRT* related gene,

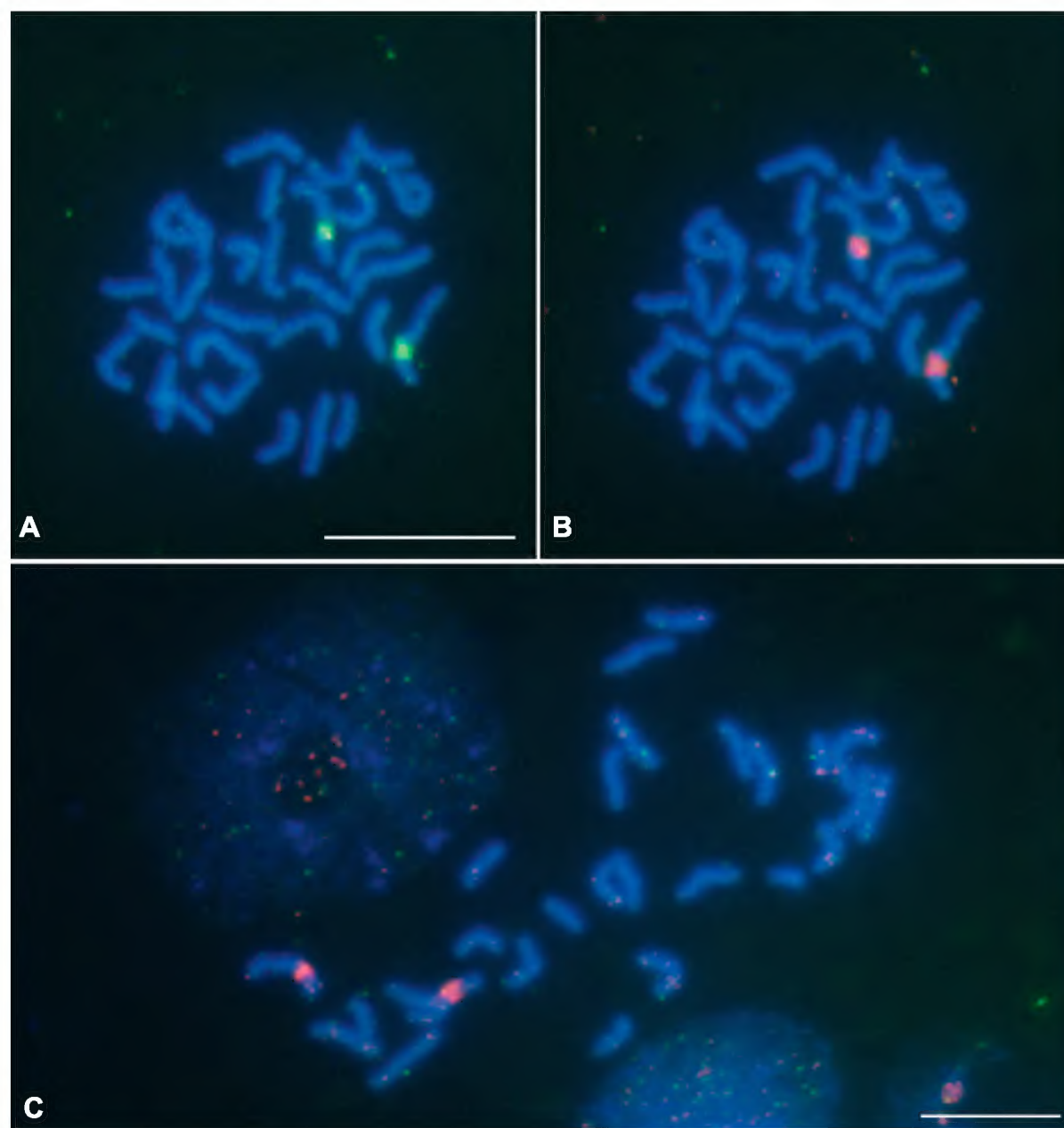


Fig. 4, A-C. FISH in *H. magnipapillata* mitotic chromosomes with *18S* and *28S* *rDNA* probes. Signals are visible in the largest chromosome pair. Chromosomes hybridized with *18S* *rDNA* probe (green signals) (A) and *28S* *rDNA* probe (red signals) (B); chromosomes hybridized with BAC probe (clone 16C18) containing *18S* *rDNA* repeats (red signals) (C). Chromosomes are counterstained blue with DAPI. Bars = 10 μ m.

DMRT-specific primers were developed to amplify the corresponding region from the *H. magnipapillata* genome. Figure 6 shows the structure of the *Hydra DMRT* gene with its 3 exons. Southern blot analysis using a 1.6 kb fragment as probe suggests that *Hydra magnipapillata* has a single *DMRT* gene (Fig. 6 B). For FISH we labelled and detected the 1.6 kb fragment of this gene (Fig. 6 A) with biotin and nanocrystals (Qdots streptavidin conjugate 655). As shown in Figure 6 (E and F), a specific signal could be discovered on one pair of middle sized chromosomes.

Can this *DMRT* signal be considered as an

evidence for initial stage of evolution of sex chromosomes in an early branching metazoan? It is known that true animal sex chromosomes should contain a region with a cluster of sex-linked genes involved in sex determination (Charlesworth, Charlesworth, 1978; Charlesworth et al., 2005). Therefore, answering this certainly fascinating question awaits both functional characterization of the *DMRT* gene (Does it trigger sex determination in the *Hydra* interstitial stem cell system?) and identifying additional sex-controlling genes indicating that this set of chromosomes indeed should be considered as “heteromorphic”.

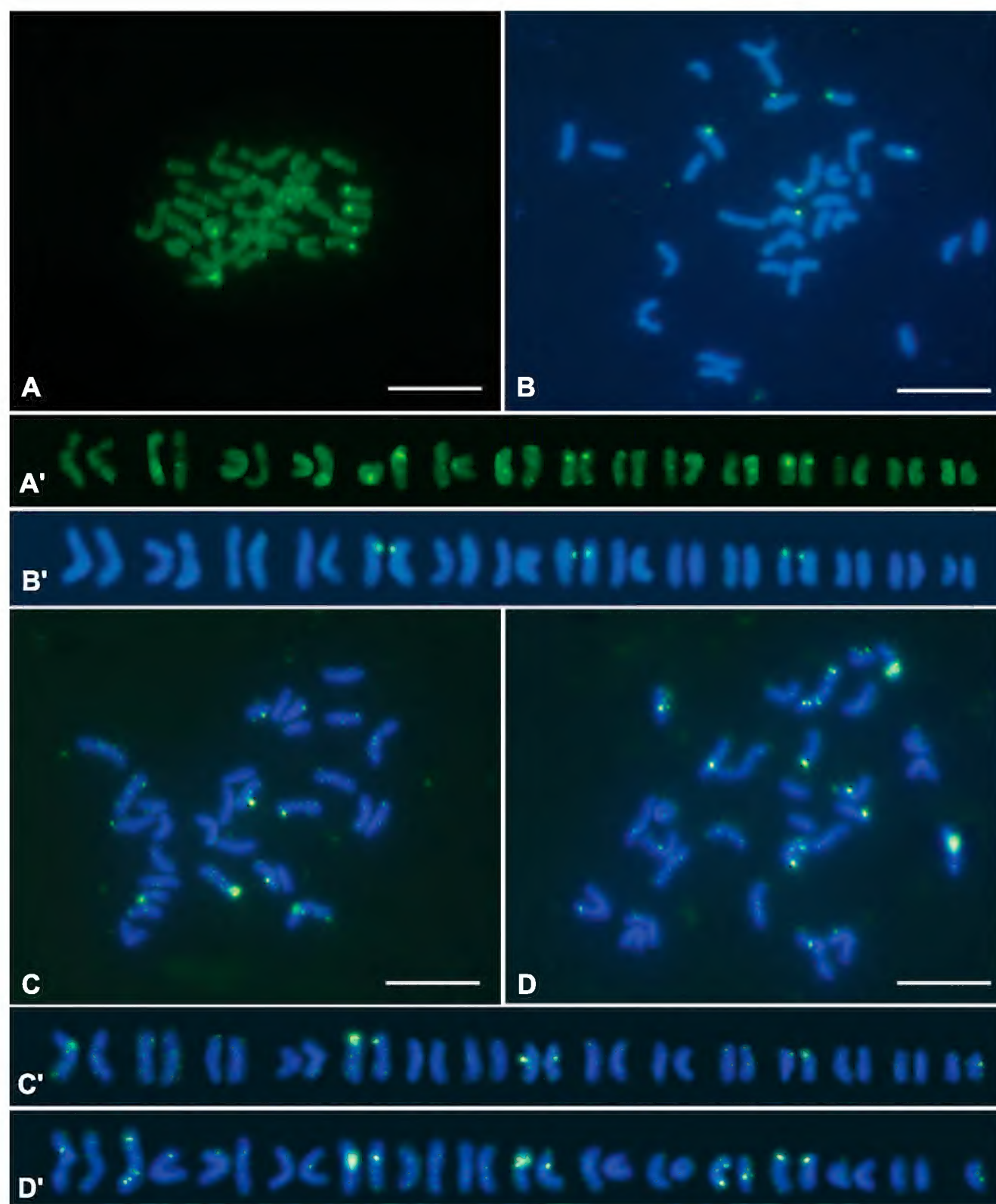


Fig. 5, A-D'. FISH in *H. magnipapillata* mitotic chromosomes with *ks1* probes. Mitotic plates (A, B) and karyograms (A', B') from different FISH experiments with 1.5 kb size probe. Signals are visible in the same chromosome pairs: 5th, 8th and 12th. Mitotic plate (C, D) and karyogram (C', D') after FISH experiment with 10 kb size BAC probe (clone 18A7) containing *ks1* gene fragment. Bright signals are visible in the chromosome pairs 5th, 8th and 12th. Additional weak signals are visible in some other chromosomes. Chromosomes are counterstained blue with DAPI. Bars = 10 μ m.

CONCLUSION AND PERSPECTIVE

We report here the localization of several genes and telomeric repeats on the chromosomes of *H. magnipapillata* using the FISH method. Telomeres signals are located in every chromosome end. Their absence in

interstitial positions may point to absence of chromosome fusions in *Hydra* karyotype evolution.

We also emphasize here that FISH allows the marking of individual chromosomes in *H. magnipapillata*. The *18S rDNA* and *28S rDNA* probes appear to mark the first

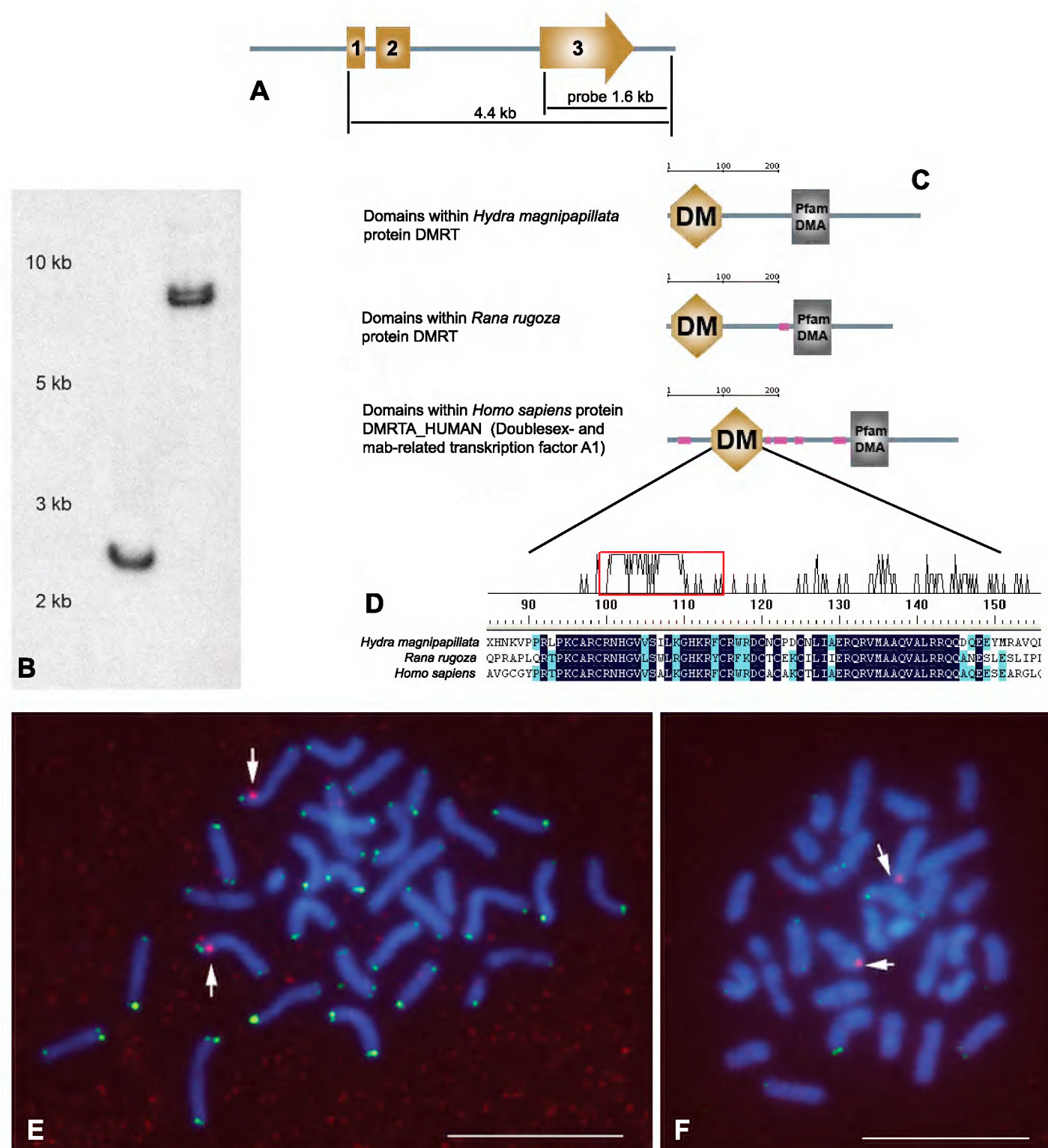


Fig. 6, A-F. Localization of the *H. magnipapillata* *DMRT* gene on metaphase spreads by two-color FISH. Schematic representation of *H. magnipapillata* *DMRT* gene and probe (**A**). Southern blot of *H. magnipapillata* DNA digested with restrictases HindIII (left lane) and XbaI (right lane) hybridized with *DMRT* probe (**B**). Domains structure of *DMRT* gene of *H. magnipapillata* in comparison with *Rana rugosa* and *Homo sapiens*. The identification and analysis of domain architecture has been done using Simple Modular Architecture Research Tool (http://smart.embl-heidelberg.de/smart/change_mode.pl) (**C**). DM domain of *DMRT* is highly conserved across distant taxa (protein alignment, DNAMAN version 4.15 was used; *H. magnipapillata* - accession No. in GenBank XM_002158684.1, *Rana rugosa* - accession No. in GenBank AB264330.1, *Homo sapiens* - accession No. in GenBank AF130728.1) (**D**). Specific hybridization signals with the *DMRT* (red signals) and a telomere specific probe (green signals) localize *DMRT* to one pair of chromosomes (**E, F**). Chromosomes are counterstained blue with DAPI. Bars = 10 μ m.

chromosome pair (Fig. 2, 4). Probes specific for the *ks1* gene family clearly mark three distinct chromosome pairs (Fig. 5). The observation makes it likely that future efforts of coupling

FISH with flow-sorting, microdissection, and genomic PCR technology could open the way to cross-species chromosome painting on a genome-wide scale. In its turn, this may allow,

for the first time, comparison of the genome architecture of basal metazoans, hence tracking ancestral genomic changes and providing an attractive tool for reconstruction of ancestral karyotypes.

Our findings also indicate that FISH in *H. magnipapillata* can detect the localization of single copy genes on *Hydra* chromosomes. Most interestingly, one sex-related gene, *DMRT*, was discovered on a single pair of chromosomes (Fig. 6). Does this indicate that *DMRT* is involved in *Hydra magnipapillata* sex determination? *Hydra*'s sex determination remains a mystery. Germ line cells continuously originate from multipotent interstitial stem cells (Bosch, David, 1987). In contrast to other invertebrates and vertebrates where the gonads are the initial determinants of sex, in *Hydra* sex determination reflects the sex of the interstitial cell lineage and is independent of the genetic sex of the epithelial (gonadal) cells (Littlefield, 1984; Campbell, 1985). We currently assume (Bosch, David, 1986) that the sexual phenotype of *Hydra* polyps is controlled by the switching rate of male and female stem cells and the repression of female differentiation by male stem cells. The molecular mechanisms controlling the determination of sex in *Hydra* and other cnidarians remain to be discovered (Fautin, 2002). Localization of *DMRT* on a single pair of *Hydra* chromosome (Fig. 6) admits the possibility of a dose-regulated testis (or ovary)-determining gene.

Taken together, we expect that application of FISH karyotyping to cross-species comparisons in the genus *Hydra* will have a considerable impact on the understanding of chromosome changes that occurred during animal evolution. Characterization of chromosomes in *Hydra* is certainly as fascinating as the unique biology of this basal metazoan model organism.

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REFERENCES

- Alexandrova O., Solovei I., Cremer T., David C.N. 2003. Replication labeling patterns and chromosome territories typical of mammalian nuclei are conserved in the early metazoan *Hydra* // *Chromosoma*. 112(4): 190-200.
- Anokhin B.A., Kuznetsova V.G. 1999. Chromosome morphology and banding patterns in *Hydra oligactis* Pallas and *H. circumcincta* Schulze (Hydroidea, Hydrida) // *Folia biol.* (Kraków). 47(3-4): 91-96.
- Anokhin B.A. 2002. Redescription of the endemic Baikal species *Pelmatohydra baikalensis* (Cnidaria: Hydrozoa, Hydrida, Hydridae) and assessment of the hydra fauna of Lake Baikal // *Ann. Zool.* (Warszawa). 52(4): 195-201.
- Anokhin B.A. 2004. Revision of Hydrida (Cnidaria, Hydrozoa): comparative morphological, karyological and taxonomical aspects. Ph.D Dissertation, Zoological Institute, Russian Academy of Sciences. St. Petersburg, 190 p. (In Russian).
- Anokhin B., Nokkala S. 2004. Characterization of C-heterochromatin in four species of Hydrozoa (Cnidaria) by sequence specific fluorochromes Chromomycin A₃ and DAPI // *Caryologia*. 57(2): 167-170.
- Augustin R., Fraune S., Bosch T.C.G. 2010. How *Hydra* senses and destroys microbes // *Seminars in Immunology*. 22: 54-58.
- Bosch T.C.G., David C.N. 1986. Male and female stem cells and sex reversal in *Hydra* polyps // *Proc.*

- Nat. Acad. Sci. USA.* 83(24): 9478-9482.
- Bosch T.C.G., David C.N. 1987.** Stem cells of *Hydra magnipapillata* can differentiate into somatic cells and germ line cells // *Dev. Biol.* 121: 182-191.
- Bosch T.C.G. 2007.** Why polyps regenerate and we don't: towards a cellular and molecular framework for *Hydra* regeneration // *Dev. Biol.* 303: 421-433.
- Bosch T.C.G. (Ed.) 2008.** Stem cells: from *Hydra* to man. Springer Netherlands. 192 p.
- Bosch T.C.G. 2009.** *Hydra* and the evolution of stem cells // *BioEssays*. 31(4): 478-486.
- Bosch T.C.G., Augustin R., Anton-Erxleben F., Fraune S., Hemmrich G., Zill H., Rosenstiel P., Jacobs G., Schreiber S., Leippe M., Stanisak M., Grötzinger J., Jung S., Podschun R., Bartels J., Harder J., Schröder J-M. 2009.** Uncovering the evolutionary history of innate immunity: the simple metazoan *Hydra* uses epithelial cells for host defence // *Dev. Comp. Immunol.* 33: 559-569.
- Bosch T.C.G., Anton-Erxleben F., Hemmrich G., Khalturin K. 2010.** The *Hydra* polyp: Nothing but an active stem cell community // *Dev. Growth. Differ.* 52(1): 15-25.
- Campbell R.D. 1985.** Sex determination in *Hydra*: roles of germ cells (interstitial cells) and somatic cells // *J. Exp. Zool.* 234: 451-458.
- Chandramore K., Ito Y., Takahashi S., Asashima M., Ghaskadbi S. 2010.** Cloning of noggin gene from hydra and analysis of its functional conservation using *Xenopus laevis* embryos // *Evol. Dev.* 12(3): 267-74.
- Chapman J.A., Kirkness E.F., Simakov O., ... Khalturin K., Hemmrich G., Franke A., Augustin R., Fraune S., ... Venter J.C., Technau U., Hobmayer B., Bosch T.C.G., Holstein T.W., Fujisawa T., Bode H.R., David C.N., Rokhsar D.S., Steele R.E. 2010.** The dynamic genome of *Hydra* // *Nature*. 464(7288): 592-596.
- Charlesworth B., Charlesworth D. 1978.** A model for the evolution of dioecy and gynodioecy // *American Natur.* 112: 975-997.
- Charlesworth D., Charlesworth B., Marais G. 2005.** Steps in the evolution of heteromorphic sex chromosomes // *Heredity*. 95: 118-128.
- Endl I., Lohmann J.U., Bosch T.C.G. 1999.** Head-specific gene expression in *Hydra*: Complexity of DNA/protein interactions at the promoter of *ks1* is inversely correlated to the head activation potential // *Proc. Nat. Acad. Sci. USA.* 96: 1445-1450.
- Fautin D.G. 2002.** Reproduction of Cnidaria // *Canadian J. Zool.* 80(10): 1735-1754.
- Gee L., Hartig J., Law L., Wittlieb J., Khalturin K., Bosch T.C., Bode H.R. 2010.** Beta-catenin plays a central role in setting up the head organizer in hydra // *Dev. Biol.* 340(1): 116-24.
- Hartl M., Mitterstiller A.M., Valovka T., Breuker K., Hobmayer B., Bister K. 2010.** Stem cell-specific activation of an ancestral myc protooncogene with conserved basic functions in the early metazoan *Hydra* // *Proc. Nat. Acad. Sci. USA.* 107(9): 4051-6.
- Khalturin K., Hemmrich G., Fraune S., Augustin R., Bosch T.C.G. 2009.** More than just orphans: are taxonomically restricted genes important in evolution? // *Trends in Genet.* 25(9): 404-413.
- Littlefield. C.L. 1984.** The interstitial cells control the sexual phenotype of heterosexual chimeras of *Hydra* // *Dev. Biol.* 102:426-432.
- Lohmann J.U., Endl I., Bosch T.C. 1999.** Silencing of developmental genes in *Hydra* // *Dev. Biol.* 214(1): 211-4.
- Marín I., Siegal M.L., Baker B.S. 2000.** The evolution of dosage-compensation mechanisms // *BioEssays*. 22 (12): 1106-1114.
- Martínez D. E. 2002.** Senescence and rejuvenation in asexual metazoans, (pp. 115–140) // *Progress in Asexual Reproduction*, R. N. Hughes (Ed.). Chichester.
- Münder S., Käsbauer T., Prexl A., Aufschnaiter R., Zhang X., Towb P., Böttger A. 2010.** Notch signalling defines critical boundary during budding in *Hydra* // *Dev. Biol.* 344(1): 331-45.
- Ojimi M.C., Isomura N., Hidaka M. 2009.** Telomerase activity is not related to life history stage in the jellyfish *Cassiopea* sp. // *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 152(2): 240-244.
- Ovanesyan I., Kuznetsova V.G. 1995.** The karyotype of *Hydra vulgaris* Pall. and the survey of the karyotype data on other Hydridae species (Cnidaria, Hydrozoa, Hydroidea, Hydrida), (pp. 95-101) // Stepanjants S.D.(Ed.), "Cnidaria. Modern and perspective investigation", *Proc. Zool. Inst. RAS*, 2. (In Russian).
- Schwarzacher T., Heslop-Harrison P. 2000.** Practical *In Situ* Hybridization. BIOS Scientific Publishers. Oxford. 203 p.
- Traut W., Szczepanowski M., Vítková M., Opitz Chr., Marec F. and Zrzavý J. 2007.** The telomere repeat motif of basal Metazoa // *Chromosome Res.*

- 15: 371-382.
- Vicoso B., Bachtrog D. 2009.** Progress and prospects toward our understanding of the evolution of dosage compensation // *Chromosome Res.* 17(5): 585-602.
- Weinziger R, Salgado LM, David CN, Bosch T.C. 1994.** Ks1, an epithelial cell-specific gene, responds to early signals of head formation in *Hydra* // *Development.* 120(9): 2511-7.
- Winkler C., Hornung U., Kondo M., Neuner C., Duschl J., Shima A., Scharl M. 2004.** Developmentally regulated and non-sex-specific expression of autosomal *DMRT* genes in embryos of the Medaka fish (*Oryzias latipes*) // *Mech. Dev.* 121(7-8): 997-1005.
- Wittlieb J., Khalturin K., Lohmann J.U., Anton-Erxleben F., Bosch T.C.G. 2006.** Transgenic *Hydra* allow in vivo tracking of individual stem cells during morphogenesis // *Proc. Nat. Acad. Sci. USA.* 103; 16: 6208-6211.
- Xinbai S., Shuwei D., Xueming F., Hongling Z. Jiaying, L. 1987.** The characteristics of *Hydra robusta* and its difference from *H. oligactis* // *Acta Zool. Sinica.* 33(2): 174-179.
- Zacharias H., Anokhin B., Khalturin K., Bosch T.C.G. 2004.** Genome sizes and chromosomes in the basal metazoan *Hydra* // *Zoology.* 107: 219-227.
- Zielke S., Bodnar A. 2010.** Telomeres and telomerase activity in scleractinian corals and *Symbiodinium* spp. // *Biol. Bull.* 218(2): 113-21.

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